C-MET overexpression, c-MET is frequently overexpressed in CRC in
malignant cell lines treated with MMC (Figure 2). Overexpression of c-MET implies that c-MET may
be a therapeutic target for CRC patients. A rationale for treatment of colorectal cancer with mitomycin C and crizotinib

A rationale for treatment of colorectal cancer with mitomycin C and crizotinib

Avital Levy1, Elena Shagisultanova2, Safoora Delhimi2, David T. Dicker1, Joanne Xu3 and Wafik S. El-Deiry1.
1Fox Chase Cancer Center, Philadelphia, PA; 2University of Colorado Denver, CO and 3Caris Life Sciences, Phoenix, AZ

Abstract

C-MET overexpression. Mitomycin C (MMC) is an anti-cancer chemotherapeutic agent, which causes DNA damage by inducing double strand breaks (DSBs) through DNA cross-linking. Tumors deficient in genes encoding for proteins involved in DNA repair such as BRCA2 show hypersensitivity to MMC. Crizotinib is a small molecule inhibitor of c-MET and ALK receptor tyrosine kinases. In the present study, we tested CRC cell lines for sensitivity to MMC plus crizotinib. CRC cell lines treated with MMC activated a DNA damage response as measured by up-regulation of H2AX. Upon BRCA2 siRNA-mediated knockdown colorectal cancer cells became more sensitive to MMC shown by cleaved-PARP as a measure of apoptosis. Crizotinib inhibited the activation of c-MET in CRC cell lines treated with Hepatocyte Growth Factor (HGF). The combination treatment of crizotinib and MMC resulted in synergistic effect.

Introduction

Colorectal cancer (CRC) is the third leading cause of cancer-related death in men and women in the United States. Microsatellite instability (MSI) is found in approximately 15% of sporadic colorectal cancers and in the majority of Lynch syndrome patients. MSI has been correlated with the mismatch repair (MMR) genes and therefore MSI-High tumors exhibit higher mutation rates than non-MSI-High tumors. We recently reported on 26 MSI-High and 558 non-MSI-High CRCs that were profiled at Caris Life Sciences (Shagisultanova et al. 2015 ASCO Annual Meeting Abstract #4). A high association of increase in BRCA2 gene mutations in microsatellite instable (MSI-H) colorectal cancer (CRC) with increased c-MET expression. Immunohistochemistry and genomic analyses were performed in the BRCA-mutant versus BRCA wild-type MSI-High tumors. BRCA2 mutations were highly enriched (50%) in MSI-High CRCs. MSI-High tumors with BRCA2 frameshift mutations had high c-MET expression. c-MET overexpression is known to be associated with aggressive metastatic CRC. We hypothesized there may be a mechanistic link between BRCA2-deficiency, double-strand breaks following DNA damage, and c-MET overexpression. Mitomycin C (MMC) is an anti-cancer chemotherapeutic agent, which causes DNA damage by inducing double strand breaks (DSBs) through DNA cross-linking. Tumors deficient in genes encoding for proteins involved in DNA repair such as BRCA2 show hypersensitivity to MMC. Crizotinib is a small molecule inhibitor of c-MET and ALK receptor tyrosine kinases. In the present study, we tested CRC cell lines for sensitivity to MMC plus crizotinib. CRC cell lines treated with MMC activated a DNA damage response as measured by up-regulation of H2AX. Upon BRCA2 siRNA-mediated knockdown colorectal cancer cells became more sensitive to MMC shown by cleaved-PARP as a measure of apoptosis. Crizotinib inhibited the activation of c-MET in CRC cell lines treated with Hepatocyte Growth Factor (HGF). The combination treatment of crizotinib and MMC resulted in synergistic effect.

Results

Efficacy of CRC cell lines treated with single drug crizotinib or MMC

Crizotinib and MMC show potent synergistic potential in a CRC xenograft model

Figure 1 – A. 26 MSI-H and 558 non MSI-H were analyzed for BRCA 1/2 status and the expression of a panel of cancer markers by IHC. The comparison between BRCA-mutant MSI to BRCA WT MSI samples revealed a correlation between mutated BRCA and high expression of the proto-oncogene c-MET. B. Representative IHC images of two cases of MSI-H CRC mutant CRC with high c-MET expression.

Figure 2 – Western blot analysis of CRC cell lines treated with +/- crizotinib or MMC or vehicle. Cleavage of PARP was monitored by western blot analysis of CRC cell lines compared to each drug alone.

Figure 3 – HT-29 and DLD-1 CRC cell lines were treated with HGF. Activation of c-MET was then measured by western blot analysis of phospho-c-MET. Treatment of the cell lines with crizotinib inhibited the phosphorylation of c-MET.

Figure 4 – Representative dose response curves of HT-29 and HCT116 cell lines with crizotinib (A) and MMC(B). C. GI50 values (μM) were determined for both MMC and crizotinib on a panel of CRC cell lines.

Figure 5 – Western blot analysis of CRC cell lines treated with +/- crizotinib or MMC or combination revealed increased level of apoptosis in the combination treatment compared to each drug alone. Apoptosis was measured by looking at PARP cleavage. H2AX levels were up-regulated in samples treated with MMC.

Figure 6 – Synergistic potential of crizotinib and MMC was evaluated by cell block-Glo assay.

Figure 7 – HT-29-luciferase subcutaneous xenograft tumors were established in female nude mice. Mice were then treated with 30mg/kg crizotinib oral gavage daily or with 2mg/kg MMC IP. on day 1, 4, 7, 10 and 13 or combination of the 2 drugs. Bioluminescence measurements of tumor burden were taken with caliper measurement and bio-luminescence imaging of tumors was recorded weekly. A. Relative tumor growth curve shows inhibition of tumor growth in the group of mice that received the drug combination in comparison to each drug alone or vehicle group. B. Tumors on day 26 of treatment. C. Representative bio-luminescence imaging of 1 mouse per group at the last day of experiment.